

Blood Sugar and Syphilis Serology Using a Single Specimen

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SCREENING TESTS for diabetes and syphilis may be performed on a single blood specimen collected in a tube containing sodium fluoride, according to results obtained in recent studies.

Since the development of the Wilkerson-Heftmann method (1) using the Hewson Clinotron for the rapid examination of blood specimens for determination of sugar content, many health departments have used this testing method in mass diabetes screening programs. A number of health departments have combined diabetes screening with mass syphilis screening programs. The development of techniques for obtaining specimens for both diabetes and syphilis tests from a single venipuncture made the combination of the two screening programs economical and acceptable.

The most commonly used technique was reported by the District of Columbia Department of Public Health, and has been called the "piggyback" method (2). In this technique, a plain Sheppard tube is used to collect the specimen for serology; the needle of a second Sheppard tube containing sodium fluoride is then inserted into the rubber sleeve of the first tube to collect the specimen for sugar determination.

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The sodium fluoride prevents coagulation and preserves the glucose level for approximately 72 hours without refrigeration (3).

The Venereal Disease Research Laboratory (VDRL) slide test (4) was generally preferred for the serologic examination, and the Wilkerson-Heftmann method was used for sugar determination.

While the "piggyback" method was acknowledged to be more economical than previously used techniques, involving two venipunctures or drawing blood into a syringe and inserting the prescribed amounts into two test tubes, it still slowed up the collection of specimens for serologic screening and added to the cost of the screening program in personnel time and equipment. In addition, there was the traumatic effect on the screenee of seeing the second specimen drawn.

In early 1957, the rapid plasma reagin (RPR) test for syphilis was developed by Portnoy, Garson, and Smith (5). During the same year, its acceptability for use as a rapid, practical, and economical screening test for syphilis was demonstrated by the Venereal Disease Branch, Communicable Disease Center, Public Health Service (6). More than 47,000 Mexican farmworkers, entering the United States through the border reception center, El Centro, Calif., were tested with the RPR test.

In the RPR test, the authors (5) utilized potassium oxalate, heparin, or potassium sequestrene as an anticoagulant. In diabetes screening programs, sodium fluoride in the approximate ratio of 10 mg. per cubic centimeter of blood has been a satisfactory anticoagulant and

Table 1. Comparative results of the RPR, VDRL, and TPCF tests, Chapel Hill data

Test	Number specimens	Reactive	Non-reactive	Diagnosed cases of syphilis			Sensitivity	Specificity
				Reactive	Non-reactive	Total		
RPR ¹ -----	97	48	49	47	2	49	96	98
VDRL ² -----	97	42	55	41	8	49	84	98
TPCF ² -----	97	45	52	45	4	49	92	100

¹ On sodium fluoride specimen. ² On conventional blood specimen.

NOTE: Sensitivity = $\frac{\text{Reactors to test, confirmed as syphilitic} \times 100}{\text{Total diagnosed as syphilitic}}$

Specificity = $\frac{\text{Nonreactors to test, confirmed as nonsyphilitic} \times 100}{\text{Total diagnosed as nonsyphilitic}}$

glucose preservative when testing is not done immediately after collecting the specimens.

In studying the need for developing practical, economical, and rapid screening programs, it became apparent that the use of a single anticoagulant and preservative for both tests would simplify the collection of specimens and minimize the cost of conducting two screening programs.

Study 1

The Venereal Disease Experimental Laboratory, Chapel Hill, N.C., cooperated in a small pilot study to determine the feasibility of using sodium fluoride as an anticoagulant for the RPR test.

Ninety-seven sets of blood specimens were collected in a venereal disease prevention and control center, approximately half from diagnosed syphilitic patients, and half from undiagnosed screenees. The "piggyback" method was used, one Sheppard tube containing sodium fluoride, and the other containing no preservative. The following day in the experimental

laboratory, the RPR test was performed on the specimens containing sodium fluoride, and the VDRL slide test and the *Treponema pallidum* complement fixation (TPCF) test (?) were performed on the conventional blood specimens. The results of the study are shown in table 1. The diagnosis of syphilis was established or ruled out for every patient by means of a clinical diagnostic workup.

The results of study 1 indicated that the sodium fluoride tube yielded an acceptable specimen for the RPR test, making it possible to do combined syphilis and diabetes testing on a single specimen. In order to confirm the findings of this small study, results of the RPR and VDRL tests were compared on a larger volume of specimens under more typical field conditions.

Study 2

The Virginia Department of Health and the Richmond City Health Department participated in a study using specimens of blood obtained from the city jail population during April, May, and June 1958.

Table 2. Comparative results of RPR and VDRL tests, Richmond data

Test	Number specimens	Reactive	Number reactors diagnosed as syphilitic	Relative sensitivity	Relative specificity
RPR ¹ -----	1, 317	156	140	98	99
VDRL ² -----	1, 317	107	100	70	99

¹ On sodium fluoride specimen. ² On conventional blood specimen.

Using the "piggyback" method, specimens were drawn into two Sheppard tubes, one of which had been prepacked with approximately 30 mg. of sodium fluoride. The conventional specimen was drawn into the plain tube, after which approximately 3 cc. of blood were drawn into the sodium fluoride tube. Specimens were examined in the laboratory of the Virginia Department of Health. The VDRL slide test was performed on the clotted specimens, and both RPR and sugar determination tests were performed on the sodium fluoride specimens.

Specimens from a total of 1,334 persons were tested by the VDRL and RPR methods. Of these, 1,157 were nonreactive to both tests and 177 were reactive to one or both tests.

Of the 177 persons with reactive test results, 143 were confirmed as having syphilis by a clinical diagnostic workup, and 17 were diagnosed as nonsyphilitic. The remaining reactors were lost to followup and therefore no definitive diagnoses are available for these individuals (11 were reactive to both tests and 6 were reactive to the RPR test only). These persons are excluded from the final computations. Comparative results on the remaining 1,317 specimens are presented in table 2. Diagnostic workups were not done on the non-reactors and since definitive diagnoses are unavailable for this group, the terms "relative" sensitivity and "relative" specificity are used.

The results of the Richmond trial tended to confirm the findings of study 1. The results indicated that the RPR test on sodium fluoride blood specimens was a more sensitive screening technique than the VDRL slide test on blood specimens collected in the usual manner.

Field Trial

Following the satisfactory results of the two controlled studies, the new method of collecting blood in the District of Columbia's diabetes and syphilis screening programs was used to replace the "piggyback" technique.

During June, July, August, and the early part of September 1958, 22,965 blood specimens were drawn, using the single prepacked sodium fluoride Sheppard tube. Specimens were tested in the District of Columbia Health Department laboratory for sugar content and serologic reac-

tion. The use of this technique proved highly successful.

It has been estimated that the reduction in the number of Sheppard tubes used and the saving of clerical and technicians' time amounted to a financial saving of at least \$7,092.65 for the collecting, processing, and reporting of the 22,965 specimens.

	<i>Savings</i>
Tubes -----	\$2,066.85
2 (GS-2) clerks-----	2,170.00
Technicians' time-----	2,755.80
Miscellaneous -----	100.00
Total-----	\$7,092.65

The D.C. Health Department laboratory reported the frequent occurrence of hemolysis. Apparently, however, this has not affected the accuracy of the results of the RPR test. Hemolysis can be reduced by gentle agitation of the tubes, and by running the RPR test as soon as possible after collecting the specimen. Extremes in temperature should be avoided and refrigeration should not be used unless specimens are to be kept more than 48 hours before testing.

Summary

Two studies were made to determine the feasibility of using sodium fluoride as an anti-coagulant in blood specimens collected for the rapid plasma reagin screening test for syphilis.

Study 1 consisted of testing two specimens each for 97 individuals. One specimen, without preservative, received the VDRL and TPCF tests; the second specimen, containing sodium fluoride, received the RPR test. The sensitivity rates for the three tests were: RPR, 96 percent; VDRL, 84 percent; and TPCF, 92 percent. The RPR and VDRL tests showed a specificity of 98 percent, and TPCF tests showed a specificity of 100 percent.

Study 2 compared results of VDRL and RPR tests on 1,317 sets of specimens (VDRL, conventional specimen; RPR, sodium fluoride specimen). The relative sensitivity of the RPR test was 98 percent, the VDRL, 70 percent. The relative specificity of the RPR test was 99 percent, the VDRL, 99 percent.

In the field trial, the District of Columbia

Health Department initiated the use of the study technique (June 1958) and reported a financial saving of at least \$7,092.65 on the collecting, processing, and reporting of the first 22,965 specimens.

The use of this technique not only simplified the collecting and handling of specimens, but it also eliminated approximately half of the clerical work; and because of the speed and ease with which the specimen was collected, minimized the traumatic effect on the screenee.

The results of these studies indicate that the sodium fluoride tube yields an acceptable specimen for the RPR test, thereby making it possible to combine syphilis and diabetes screening programs using a single blood specimen for both tests.

The method is recommended as acceptable, practical, and economical for use wherever it is desirable to do syphilis and diabetes screening on the same population group.

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Milwaukee's Fluoridation Reduces Caries

A dental examination of 4,660 school children conducted by the Milwaukee Health Department following 6 years of fluoridation of Milwaukee's water supply revealed a significant reduction in the incidence of dental decay in all age groups, 5 through 13 years. Fluoridation levels were 0.9 parts per million in the winter and 0.7 parts per million in the summer.

The DMF index for a 7-year-old child was 1.29 prior to fluoridation and only 0.53 after 6 years of fluoridation, a reduction of some 59 percent in the amount of dental decay.

After fluoridation 8-year-old children showed decay in 46.9 percent of their 6-year molars; prior to fluoridation the comparable figure was 81.1 percent. Of the 6-year-old children entering the first grade this year, 31.0 percent were free from caries in their deciduous teeth; before fluoridation the percentage was 20.8.

During the 6-year period, the total cost of fluoridation was \$240,468. The total saving in dental care necessary for permanent teeth was approximately \$718,164. The annual per capita cost of fluoridation was estimated at 5½ cents.

A second study is projected 6 years from now after 12 years of fluoridation of Milwaukee's water supply.